Figure 1

Gene symbol	Gene name
OSBP	Oxysterol-binding protein
NFKB3 (p65)	nuclearfactor of kappa light polypeptide gene enhancer B-cells 3
CAPN1	Calpain, large polypeptide L1
CCND1	Cyclin D1
EFEMP2*	EGF-containing fibulin-like extracellular matrix protein 2
FOSL1	FOS-like antigen-1
PLCB3	Phospholipase C, beta 3 (phosphatidylinositol-specific)
PPP1CA	Protein phosphatase-1, catalytic subunit, alpha isoform
VEGFB	Vascular endothelial growth factor B
ESRRA, ERR1	estrogen-related receptor alpha
CTSW	Cathepsin W
GALN*	Galanin
LRP5, LRP7, LR3*	Low density lipoprotein receptor-related protein-5
CBP2	Collagen-binding protein 2 (colligen 2)
TCIRG1	T-cell immune regulator 1
LTBP3	Latent transforming growth factor-beta binding protein-3
FGF19*	Fibroblast growth factor 19 (FGF19)
Delta5-desaturase*	Delta5-desaturase

Figure 2

GCCATGGAGCCCGAGTGAGCGCGGCGCGGGCCCGTCCGGCCGCCGGACAACAT
GGAGGCAGCGCCGCCCGGGCCGCCGTGGCCGCTGCTGCTGCTGCTGCTGCTGCT
GCTGGCGCTGTGCGGCTGCCCGGCCCCCGCCGCGGCCTCGCCGCTCCTGCTATTT
GCCAACCGCCGGGACGTACGGCTGGTGGACGCCGGCGGAGTCAAGCTGGAGTC
CACCATCGTGGTCAGCGGCCTGGAGGATGCGGCCGCAGTGGACTTCCAGTTTTC
CAAGGGAGCCGTGTACTGGACAGACGTGAGCGAGGAGGCCATCAAGCAGACCT
ACCTGAACCAGACGGGGGCCGCCGTGCAGAACGTGGTCATCTCCGGCCTGGTCT
CTCCCGACGGCCTCGCCTGCGACTGGGTGGGCAAGAAGCTGTACTGGACGGACT
CAGAGACCAACCGCATCGAGGTGGCCAACCTCAATGGCACATCCCGGAAGGTGC
TCTTCTGGCAGGACCTTGACCAGCCTAGGGCCATCGCCTTGGACCCCGCTCACG
GGTACATGTACTGGACAGACTGGGGTGAGACGCCCCGGATTGAGCGGGCAGGG
ATGGATGGCAGCACCCGGAAGATCATTGTGGACTCGGACATTTACTGGCCCAAT
GGACTGACCATCGACCTGGAGGAGCAGAAGCTCTACTGGGCTGACGCCAAGCTC
AGCTTCATCCACCGTGCCAACCTGGACGGCTCGTTCCGGCAGAAGGTGGTGGAG
GGCAGCCTGACGCACCCCTTCGCCCTGACGCTCTCCGGGGACACTCTGTACTGG
ACAGACTGGCAGACCCGCTCCATCCATGCCTGCAACAAGCGCACTGGGGGGAAG
AGGAAGGAGATCCTGAGTGCCCTCTACTCACCCATGGACATCCAGGTGCTGAGC
CAGGAGCGGCAGCCTTTCTTCCACACTCGCTGTGAGGAGGACAATGGCGGCTGC
TCCCACCTGTGCCTGCTGTCCCCAAGCGAGCCTTTCTACACATGCGCCTGCCCCA
CGGGTGTGCAGCTGCAGGACAACGGCAGGACGTGTAAGGCAGGAGCCGAGGAG
GTGCTGCTGCTGGCCCGGCGGACGGACCTACGGAGGATCTCGCTGGACACGCCG
GACTTCACCGACATCGTGCTGCAGGTGGACGACATCCGGCACGCCATTGCCATC
GACTACGACCCGCTAGAGGGCTATGTCTACTGGACAGATGACGAGGTGCGGGCC
ATCCGCAGGGCGTACCTGGACGGGTCTGGGGCGCAGACGCTGGTCAACACCGAG
ATCAACGACCCCGATGGCATCGCGGTCGACTGGGTGGCCCGAAACCTCTACTGG
ACCGACACGGGCACGGACCGCATCGAGGTGACGCGCCTCAACGGCACCTCCCGC
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GTGATGGGCCTCATGTACTGGACAGACTGGGGAGAGAACCCTAAAATCGAGTGT
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CCCAACGGCCTGGCCCTGGACCTGCAGGAGGGGAAGCTCTACTGGGGAGACGCC
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CCTGGAGGACAAGCTCCCGCACATTTTCGGGTTCACGCTGCTGGGGGACTTCAT
CTACTGGACTGACTGGCAGCGCCGCAGCATCGAGCGGGTGCACAAGGTCAAGGC
CAGCCGGGACGTCATCATTGACCAGCTGCCCGACCTGATGGGGCTCAAAGCTGT
GAATGTGGCCAAGGTCGTCGGAACCAACCCGTGTGCGGACAGGAACGGGGGGT
GCAGCCACCTGTGCTTCTTCACACCCCACGCAACCCGGTGTGGCTGCCCCATCG
GCCTGGAGCTGCTGAGTGACATGAAGACCTGCATCGTGCCTGAGGCCTTCTTGG
TCTTCACCAGCAGAGCCGCCATCCACAGGATCTCCCTCGAGACCAATAACAACG
ACGTGGCCATCCCGCTCACGGGCGTCAAGGAGGCCTCAGCCCTGGACTTTGATG
TGTCCAACAACCACATCTACTGGACAGACGTCAGCCTGAAGACCATCAGCCGCG
CCTTCATGAACGGGAGCTCGGTGGAGCACGTGGTGGAGTTTGGCCTTGACTACC
CCGAGGGCATGGCCGTTGACTGGATGGGCAAGAACCTCTACTGGGCCGACACTG
GGACCAACAGAATCGAAGTGGCGCGGCTGGACGGGCAGTTCCGGCAAGTCCTC
GTGTGGAGGGACTTGGACAACCCGAGGTCGCTGGCCCTGGATCCCACCAAGGGC
TACATCTACTGGACCGAGTGGGGCGGCAAGCCGAGGATCGTGCGGGCCTTCATG

GACGGGACCAACTGCATGACGCTGGTGGACAAGGTGGGCCGGGCCAACGACCT
CACCATTGACTACGCTGACCAGCGCCTCTACTGGACCGACCTGGACACCAACAT
GATCGAGTCGTCCAACATGCTGGGTCAGGAGCGGGTCGTGATTGCCGACGATCT
CCCGCACCCGTTCGGTCTGACGCAGTACAGCGATTATATCTACTGGACAGACTG
GAATCTGCACAGCATTGAGCGGGCCGACAAGACTAGCGGCCGGAACCGCACCCT
CATCCAGGGCCACCTGGACTTCGTGATGGACATCCTGGTGTTCCACTCCTCCCGC
CAGGATGGCCTCAATGACTGTATGCACAACAACGGGCAGTGTGGGCAGCTGTGC
CTTGCCATCCCCGGCGGCCACCGCTGCGGCTGCGCCTCACACTACACCCTGGAC
CCCAGCAGCCGCAACTGCAGCCCGCCCACCACCTTCTTGCTGTTCAGCCAGAAA
TCTGCCATCAGTCGGATGATCCCGGACGACCAGCACAGCCCGGATCTCATCCTG
CCCCTGCATGGACTGAGGAACGTCAAAGCCATCGACTATGACCCACTGGACAAG
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GACCCAGCCCTTTGTTTTGACCTCTCTGAGCCAAGGCCAAAACCCAGACAGGCA
GCCCCACGACCTCAGCATCGACATCTACAGCCGGACACTGTTCTGGACGTGCGA
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GGGTACCTGTACTTCACCAACATGCAGGACCGGGCAGCCAAGATCGAACGCGCA
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GTGGCCCTGGTGGTGGACAACACACTGGGCAAGCTGTTCTGGGTGGACGCGGAC
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GACGCCAACATCGTGCAGCCTCTGGGCCTGACCATCCTTGGCAAGCATCTCTAC
TGGATCGACCGCCAGCAGCAGATGATCGAGCGTGTGGAGAAGACCACCGGGGA
CAAGCGGACTCGCATCCAGGGCCGTGTCGCCCACCTCACTGGCATCCATGCAGT
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CTGTACCCGCCGATCCTGAACCCGCCGCCCTCCCCGGCCACGGACCCCTCCCTG
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AAATATAATTGGGATTTTAAAAACATGAGAAATGTGAACTGTGATGGGGTGGGC
AGGGCTGGGAGAACTTTGTA

Figure 3

MEAAPPGPPWPLLLLLLLLLALCGCPAPAAASPLLLFANRRDVRLVDAGGVKLESTI
VVSGLEDAAAVDFQFSKGAVYWTDVSEEAIKQTYLNQTGAAVQNVVISGLVSPDGL
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TDWGETPRIERAGMDGSTRKIIVDSDIYWPNGLTIDLEEQKLYWADAKLSFIHRANL
DGSFRQKVVEGSLTHPFALTLSGDTLYWTDWQTRSIHACNKRTGGKRKEILSALYSP
MDIQVLSQERQPFFHTRCEEDNGGCSHLCLLSPSEPFYTCACPTGVQLQDNGRTCKA
GAEEVLLLARRTDLRRISLDTPDFTDIVLQVDDIRHAIAIDYDPLEGYVYWTDDEVR
AIRRAYLDGSGAQTLVNTEINDPDGIAVDWVARNLYWTDTGTDRIEVTRLNGTSRK
ILVSEDLDEPRAIALHPVMGLMYWTDWGENPKIECANLDGQERRVLVNASLGWPN
GLALDLQEGKLYWGDAKTDKIEVINVDGTKRRTLLEDKLPHIFGFTLLGDFIYWTD
WQRRSIERVHKVKASRDVIIDQLPDLMGLKAVNVAKVVGTNPCADRNGGCSHLCFF
TPHATRCGCPIGLELLSDMKTCIVPEAFLVFTSRAAIHRISLETNNNDVAIPLTGVKE
ASALDFDVSNNHIYWTDVSLKTISRAFMNGSSVEHVVEFGLDYPEGMAVDWMGKN
LYWADTGTNRIEVARLDGQFRQVLVWRDLDNPRSLALDPTKGYIYWTEWGGKPRI
VRAFMDGTNCMTLVDKVGRANDLTIDYADQRLYWTDLDTNMIESSNMLGQERVV
IADDLPHPFGLTQYSDYIYWTDWNLHSIERADKTSGRNRTLIQGHLDFVMDILVFHS
SRQDGLNDCMHNNGQCGQLCLAIPGGHRCGCASHYTLDPSSRNCSPPTTFLLFSQKS
AISRMIPDDQHSPDLILPLHGLRNVKAIDYDPLDKFIYWVDGRQNIKRAKDDGTQPF
VLTSLSQGQNPDRQPHDLSIDIYSRTLFWTCEATNTINVHRLSGEAMGVVLRGDRDK
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KLFWVDADLKRIESCDLSGANRLTLEDANIVQPLGLTILGKHLYWIDRQQQMIERVE
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SCPVHLVLLQNLLTCGEPPTCSPDQFACATGEIDCIPGAWRCDGFPECDDQSDEEGC
PVCSAAQFPCARGQCVDLRLRCDGEADCQDRSDEADCDAICLPNQFRCASGQCVLI
KQQCDSFPDCIDGSDELMCEITKPPSDDSPAHSSAIGPVIGIILSLFVMGGVYFVCQRV
VCQRYAGANGPFPHEYVSGTPHVPLNFIAPGGSQHGPFTGIACGKSMMSSVSLMGG
RGGVPLYDRNHVTGASSSSSSSTKATLYPPILNPPPSPATDPSLYNMDMFYSSNIPAT
ARPYRPYIIRGMAPPTTPCSTDVCDSDYSASRWKASKYYLDLNSDSDPYPPPPTPHSQ
YLSAEDSCPPSPATERSYFHLFPPPPSPCTDSS

Figure 4

Source	Nucleotide change	Protein change	Putative effect
OPS 88	G29A	Trp10stop	Truncation
Control	A459G	Pro153Pro	No change
Control	InsCTG33	Insert Leu at residue 12	Alters signal peptide
OPS 78	GACCTACG 1051-1058 ACCCTACA	AspLeuSer 351-353 ThrLeuLys	Unknown
OPS 59	C1282T	Arg428stop	Truncation
OPS 53	G1253T	Glu485stop	Truncation
OPS 23	delG1467	Frameshift	Truncation
OPS 82	G1481A	Arg494Gln	Unknown
OPS 2	C1708T	Arg570Trp	Unknown
OPS 72	G1999A	Val677Met	Unknown
OPS 45	insT2150	Frameshift	Truncation
OPS 41	G2202A	Trp734stop	Unknown
Control	C2220T	Asn740Asn	Unknown
OPS 92	delG2305	Frameshift	Truncation
OPS13	C2557T	Gln853stop	Truncation
OPS 7	delA3804	Frameshift	Truncation
OPS 53	C3989T	Ala1330Leu	Unknown
OPS 72	C3989T	Ala1330Leu	Unknown
Control	G4416T	Leu1472Leu	No change

Figure 5

NAME	SEQUENCE	LOCATION	PRODUCT SIZE
LRGEN1F	5'-TTG CTG CCC TAG ACT TAG CC-3'	-119	406
LRGEN1R	5'-CCA AGT CGC TTC CGA GAC-3'	+106	
LRGEN2F	5'-CAT CCC AGG GCT GTG TAT CT-3'	-65	543
LRGEN2R	5'-ACT TGG GCT CAT GCA AAT TC-3'	+81	
LRGEN3F1	5'-CCG ATG GGT GAG ATT TTA GG-3'	-118	329
LRGEN3R1	5'-CGT GGG TAC CTA CCG GAA C-3'	+16	
LRGEN4NF	5'-TAA TTG GGT CAG CAG CAA TG-3'	-72	277
LRGEN4NR	5'-GCA CTC ACA GAA AGG CTG-3'	+8	
LRGEN5NF	5'-AGT GAC GGT CCT CTT CTG GA-3'	-51	302
LRGEN5NR	5'-CAA GTG GAT CAT TTC GAA CG-3'	+120	
LRGEN6F	5'-TGG CTG AGT ATT TCC CTT GC-3'	-95	577
LRGEN6R	5'-CCA GAA TGA CAG GTC CAG GT-3'	+85	
LRGEN7F1	5'-TGC TTC TTC TCC AGC CTC AT-3'	-14	302
LRGEN7R1	5'-ATG TGG CCA AAT AGC AGA GC-3'	+116	
LRGEN8F	5'-GCA TTG AAC CCG TCT TGT TT-3'	-109	426
LRGEN8R	5'-GGC ACC TGA GCT CAA CAC TT-3'	+100	
LRGEN9F	5'-TGC TGG GCT GTT GT GTT TA-3'	-47	407
LRGEN9R	5'-CTT TGA GGC AGG AAC AGA GG-3'	+70	
LRGEN10F	5'-AGC GAA ACT CCG TCT CAA AA-3'	-79	417
LRGEN10R	5'-GCT CTA ATC ACT GAG GGC CA-3'	+110	
LRGEN11F1	5'-GAG GGC TGA GCT GAA GAG GT-3'	-105	398
LRGEN11R1	5'-CAG GTT GGG GAA CTT GCA G-3'	+108	
LRGEN12F	5'-ATT CAT GTG GTC GCT AGG CT-3'	-113	479
LRGEN12R	5'-GAA GCT CCT TTC AGC GTC AG-3'	+40	
LRGEN13F	5'-CCA GCT CCT CTG TGG CTT AC-3'	-57	352
LRGEN13R	5'-TCC TCC CTC TGC TAA GGA CA-3'	+95	
LRGEN14F	5'-CAG AGC TCT CCA GCC AGT G-3'	-149	440
LRGEN14R	5'-CTG TGA GAG GCT GGC ATT C-3'	+82	
LRGEN15NF	5'-ATG TGA CCT GTC AGC CTC G-3'	-131	415
LRGEN15NR	5'-TGC TGC CAT TAC TGA CAA TGA-3'	+83	
LRGEN16F	5'-TCT GTC CTC CCA AGC TGA GT-3'	-76	374
LRGEN16R	5'-CAC ACA GGA TCT TGC ACT GG-3'	+88	
LRGEN17F	5'-CAT GAG TTC TCA TTT GGC CC-3'	-92	321
LRGEN17R	5'-GCC ACA GGG ACT GTG ATT TT-3'	+103	
LRGEN18F	5'-CAA CTT CTG CTT TGA AGC CC-3'	-88	423
LRGEN18R	5'-CAG AGC CCC TAC TCC TGT GA-3'	+98	
LRGEN19F	5'-CCA GAC CTT GGT TGC TGT G-3'	-81	269
LRGEN19R	5'-CGT CTC CTC CCC TAA ACT CC-3'	+77	
LRGEN20NF	5'-ATG TTG GCC ACC TCT TTC TG-3'	-34	310
LRGEN20NR	5'-CTG CCT CCT CCA GAT CAT TC-3'	+39	
LRGEN21F	5'-GAG TCT CGT GGG TAG TGG GA-3'	-102	373

LRGEN21R	5'AGA AAG CAA GCA TGC CTC AG-3'	+131	
LRGEN22F	5'-AGC CCT CTC TGC AAG GAA AG-3'	-96	305
LRGEN22R	5'-GCC CAC TAG CAC CCA GAA TA-3'	+111	
LRGEN23F	5'-GAC AGG CCT TTC CCG TTC-3'	-95	650
LRGEN23R	5'-CAG GAG GAC TCT CAT GGT GG-3'	+106	
LRCOD1F	5'-TTC GTC ATG GGT GGT GTC TA-3'	4192	416
LRCOD1R	5'-TTC CTC GAA TGA TGT AGG GC-3'	4607	
LRCOD2F	5'-ACC TGG ACT TCG TGA TGG AC-3'	2654	466
LRCOD2R	5'-CAG AAC AGT GTC CGG CTG TA-3'	3119	
LRCOD3F	5'-CCA TGG AGC CCG AGT GAG-3'	-50	504
LRCOD3R	5'-GTC AAG GTC CTG CCA GAA GA-3'	453	
LRCOD4F	5'-GGG CAA GAA GCT GTA CTG GA-3'	354	500
LRCOD4R	5'-TGG ATG TCC ATG GGT GAG TA-3'	853	
LRCOD5F	5'-CAG ACC CGC TCC ATC CAT-3'	767	484
LRCOD5R	5'-TCG TTG ATC TCG GTG TTG AC-3'	1250	
LRCOD6F	5'-ATC GAC TAC GAC CCG CTA GA-3'	1132	546
LRCOD6R	5'-GTA GAT GAA GTC CCC CAG CA-3'	1677	
LRCOD7F	5'-GCC AAG ACA GAC AAG ATC GAG-3'	1564	505
LRCOD7R	5'-TGT GGT TGT TGG ACA CAT CA-3'	2068	
LRCOD8F	5'-CAC AGG ATC TCC CTC GAG AC-3'	1966	522
LRCOD8R	5'-CTC GAT CAT GTT GGT GTC CA-3'	2487	
LRCOD9F	5'-CAG CCC TTT GTT TTG ACC TC-3'	3025	484
LRCOD9R	5-TCC AGT AGA GAT GCT TGC CA-3'	3508	
LRCOD10F	5'-AAG CGC ATT GAG AGC TGT G-3'	3400	480
LRCOD10R	5'-CTC CTC GTC GCT CTG GTC-3'	3879	
LRCOD11F	5'-CAC AGG GGA GAT CGA CTG TAT-3'	3801	480
LRCOD11R	5'-ACA TAC TCG TGC GGG AAG G-3'	4280	
LRCOD12F	5'-GTC CAG CAG CTC GTC CAG-3'	4446	567
LRCOD12R	5'-TAC AAA GTT CTC CCA GCC CT-3'	5012	
LRCOD13F	5'-TCA TGG ACG GGA CCA ACT-3'	2369	431
LRCOD13R	5'-GGT GTA GTG TGA GGC GCA G-3'	2799	

Figure 6

BMSR Construct Information

The following BSMR expression constructs have been constructed using the pcDNA3 expression vector for use in following BSMR expression, function, and other biological (e.g. ligand and downstream signaling) interactions.

1. A full length wild type construct extending from primers LRCOD3F to LRCOD12R.

Sequence encoding a FLAG antibody epitope (GAC TAC AAG GAC GAC GAT GAC AAG) was inserted into the wild-type construct immediately downstream of nucleotide 165 (relative to the "A" in the ATG translation start site). This construct expresses a BSMR protein which has a FLAG epitope between wild type BSMR amino acid residues 55 and 56.

This was accomplished using the following primer sequences and a Quickchange reaction:

LRPFLAGF: 5'-GAC TAC AAG GAC GAC GAT GAC AAG ACC ATC GTG GTC AGC GGC CTG-3'

LRPFLAGR:5'-CTT GTC ATC GTC GTC CTT GTA GGA CTC CAG CTT GAC TCC GCC-3'

Sequence encoding a MYC antibody epitope (GAG CAG AAG CTG ATA TCC GAG GAG GAC CTG) was inserted immediately uptream of the stop codon after residue 4845 (relative to the "A" in the ATG translation start site). The construct expresses a BSMR protein which has a MYC epitope at the end of the wild type BSMR polypeptide.

This was accomplished using the following primer sequences and a Quickchange reaction:

LRPMYCF: 5'-GAG CAG AAG CTG ATA TCC GAG GAG GAC CTG TGA CCT CGG CCG GGC-3'

LRPMYCR: 5'-CAG GTC CTC CTC GGA TAT CAG CTT CTG CTC GGA TGA GTC CGT GCA-3'

A expresssion construct containing both the FLAG and MYC antibody epitopes at the aforementioned sites has also been produced.

Figure 7

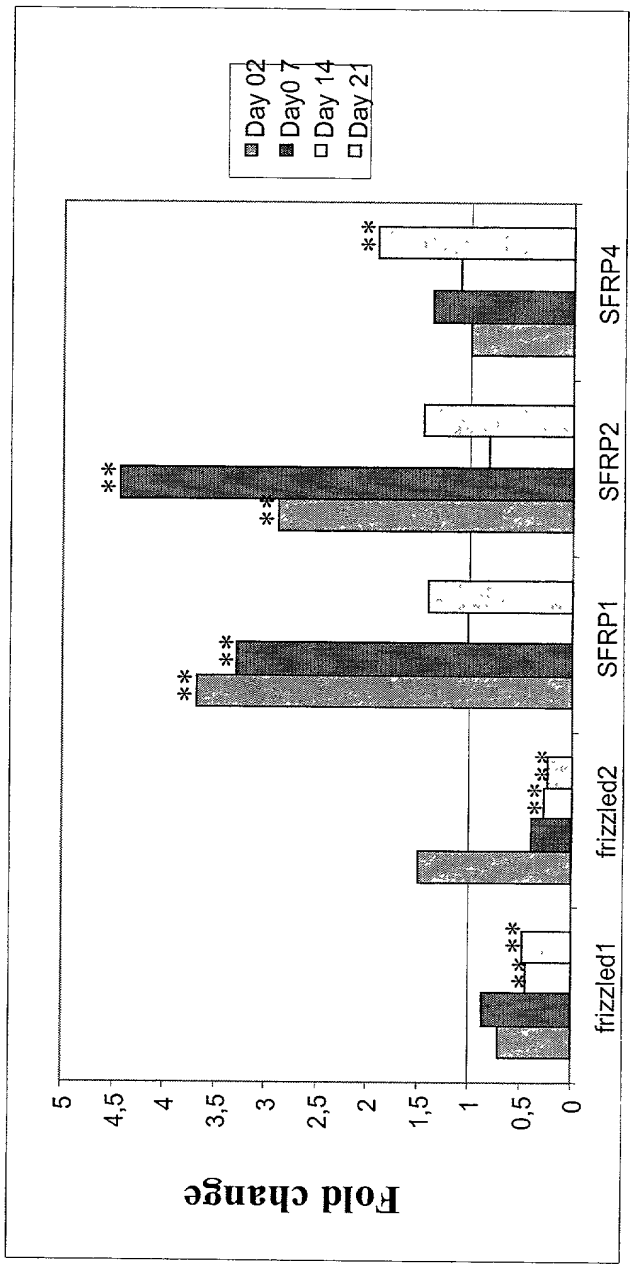
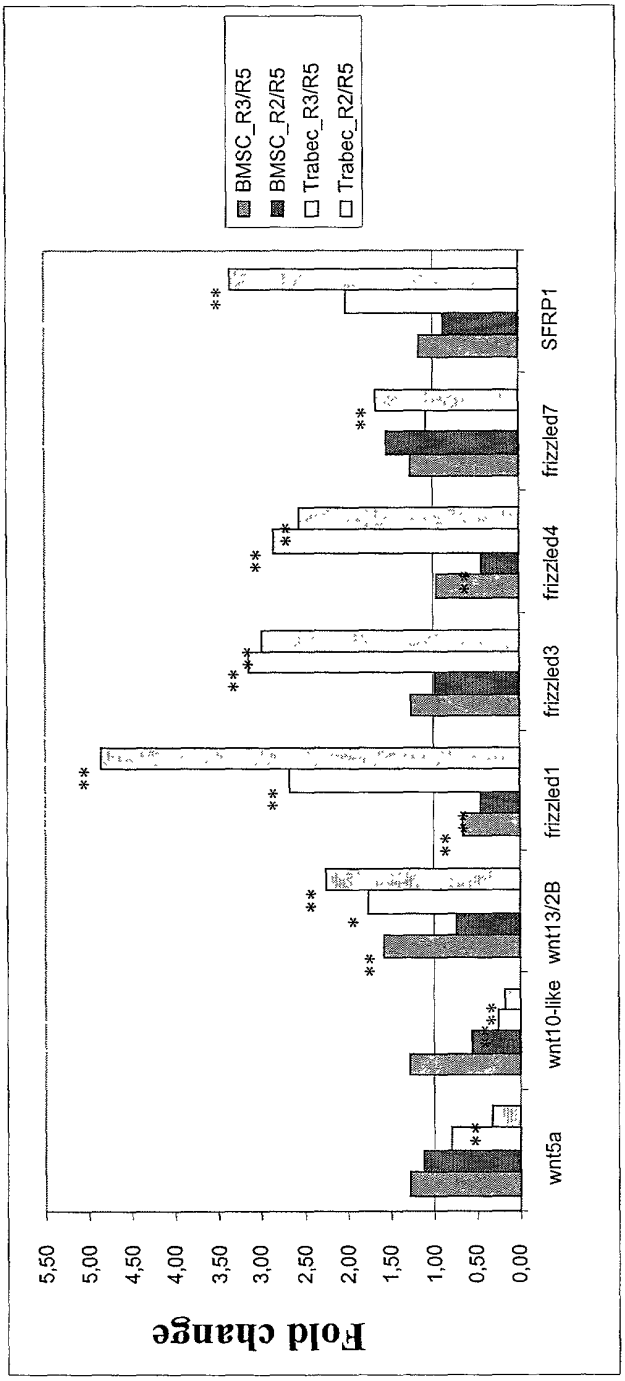

Day 02
Day0 7
Day 14
Day 21
Fold change
5
4,5
4
3,5
3
2,5
2
1,5
1
0,5
0
frizzled1
frizzled2
SFRP1
SFRP2
SFRP4

Figure 8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BMSC_R2/R5
Trabec_R3/R5
Trabec_R2/R5
Fold change
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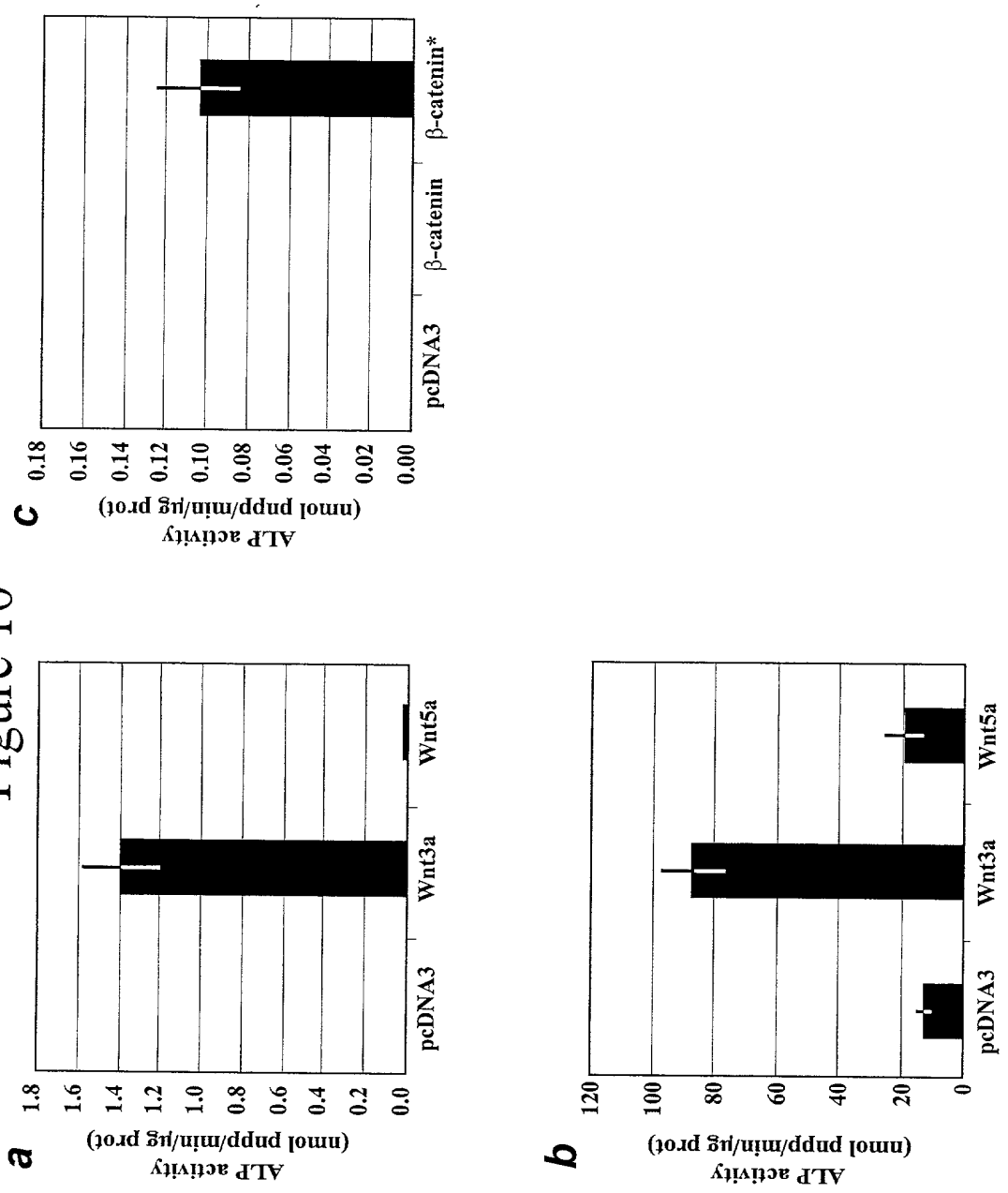
4,00
3,50
3,00
2,50
2,00
1,50
1,00
0,50
0,00
wnt5a
wnt10-like
wnt13/2B
frizzled1
frizzled3
frizzled4
frizzled7
SFRP1

Figure 9

Cell line/treatment / Gene name	C2C12/BMP2 (4 days)	C3H10T1/2/BMP2+SHH (4 days)	ST2/BMP2 (4days)	MC3T3-E1 (3days)
Frizzled-1	2.25 x	NR	2.62x	2.11x
Frizzled-4	NR	4.37x	NR	NR
SFRP2/SARP1	8.10 x	0.5x (0.4x with SHH alone)	8.54x	3.61x (0.09 with TGFbeta)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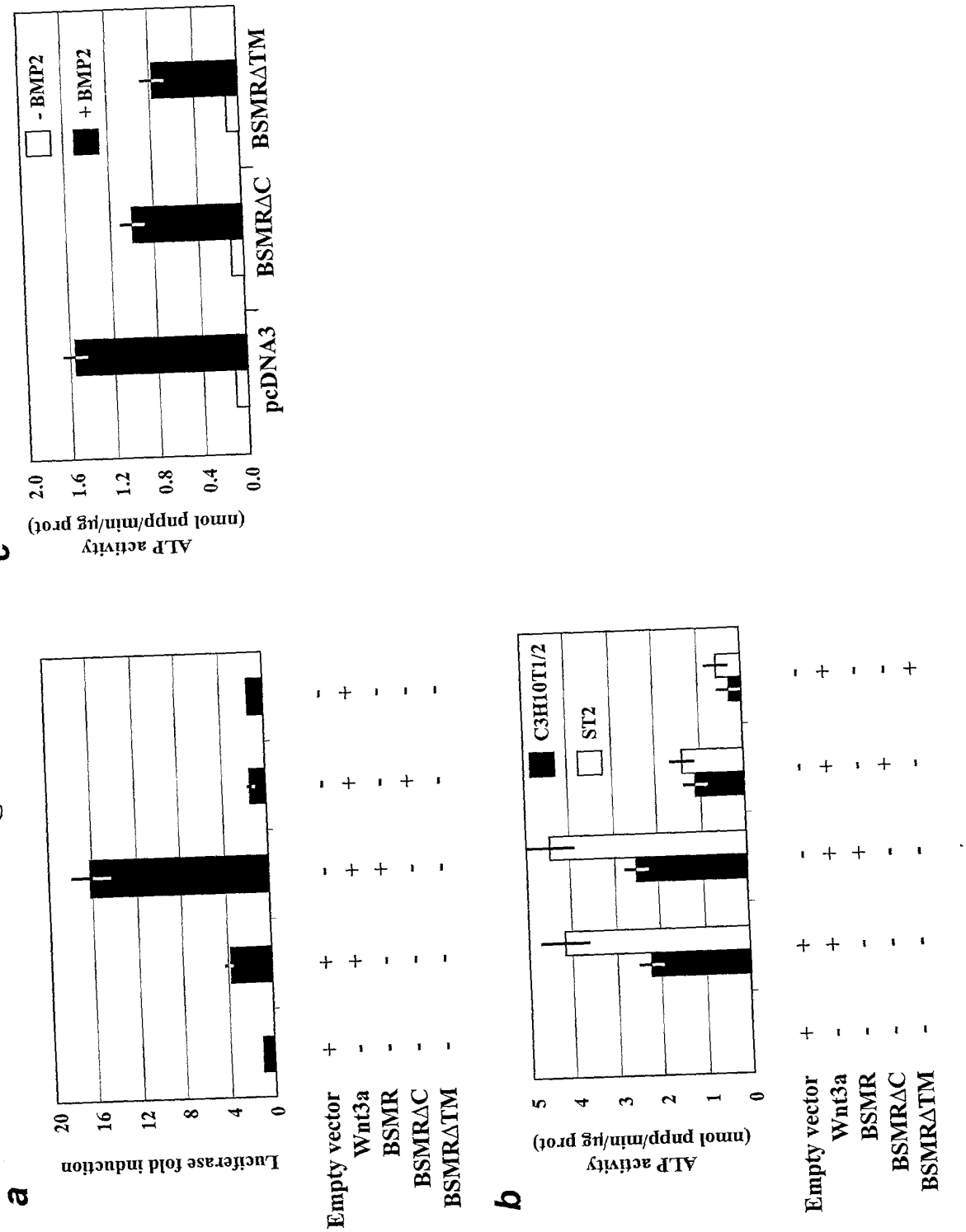
ALP activity
(nmol pnpp/min/μg prot)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
pcDNA3
Wnt3a
Wnt5a
b
ALP activity
(nmol pnpp/min/μg prot)
0
20
40
60
80
100
120
pcDNA3
Wnt3a
Wnt5a
c
ALP activity
(nmol pnpp/min/μg prot)
0.00
0.02
0.04
0.06
0.08
0.10
0.12
0.14
0.16
0.18
pcDNA3
β-catenin
β-catenin*

Figure 11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Luciferase fold induction
20
16
12
8
4
0
Empty vector
Wnt3a
BSMR
BSMRΔC
BSMRΔTM
b
ALP activity
(nmol pnpp/min/μg prot)
C3H10T1/2
ST2
5
4
3
2
1
0
Empty vector
Wnt3a
BSMR
BSMRΔC
BSMRΔTM
c
ALP activity
(nmol pnpp/min/μg prot)
- BMP2
+ BMP2
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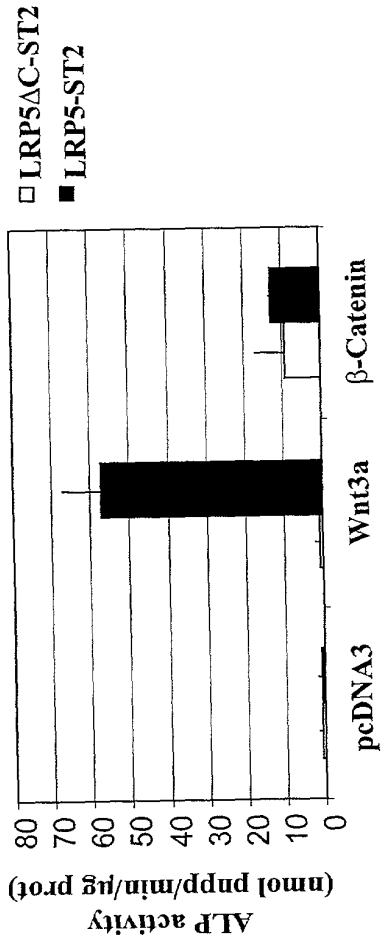
1.6
1.2
0.8
0.4
0.0
pcDNA3
BSMRΔC
BSMRΔTM

Figure 12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■LRP5-ST2
ALP activity
(nmol pnpp/min/μg prot)
0
10
20
30
40
50
60
70
80
pcDNA3
Wnt3a
β-Catenin

Figure 13

RVRLASHLRKLRK

RLTRKRGLKLA

CRAKRNNFKSA

LKWKS

KIRVKAGETQKKVIFCSREKVSHL

FIPLKPTVKMLERSNHVSRTEVSSNHV

DKGMAPALRHLYKELMGPWN

DALKLAIDNALSIT